The Influence of Adrenalin, Modified By Salt Solutions, on Blood-Pressure of the Frog and Cat

by Ivan Roy Burket

1911

Submitted to the Department of Physiology of the University of Kansas in partial fulfillment of the requirements for the Degree of Master of Arts

THE INFLUENCE OF ADRENALIN, MODIFIED BY SALT SOLUTIONS, ON BLOOD-PRESSURE OF THE FROG AND THE CAT.

BY IVAN ROY BURKET, A. B., '10.

THE INFLUENCE OF ADRENALIN, MODIFIED BY SALT SOLUTIONS, ON BLOOD PRESSURE OF THE FROG AND THE CAT.

By Ivan Roy Burket.
From the laboratory of the Physiology Department, University of Kansas.

The technique for obtaining blood-pressure in the frog is much simpler than that required in the determination of the blood-pressure in mammals. It was, therefore, undertaken to ascertain the relative merits of the two methods in the standardization of adrenalin. This was done in special reference to some work which is at present being conducted in this University by E.R.Weidlein on the suprarenal gland of the whale; this research has not progressed far enough, however, to justify publication, hence this article will be confined to results obtained from use of Parke, Davis and Co's adrenalin base. In this connection, also, the attempt was made to determine whether or not the action of adrenalin in the body is modified by the presence of various organic and inorganic salts, which may either be present normally in the blood or be otherwise used therapeutically.

Very little work has been done on the blood-pressure in the frog except to determine the effect of external influences. Schulz(1), in his paper on the effect of temperature vagus stimulation etc., made some assertions which I have been unable to corroborate and which I will discuss later; his method of operating, however, is the

(1) Schulz: Arch. f. d. ges. Phys., 1906,

one I found to be most convenient and practical and is used thruout my experiments.

Neither is literature very extensive on the influence of solutions of various salts on mammalian blood-pressure; a few experimenters, however, have published articles along this line of research, and these will be referred to in the discussions of the several salts.

I have been able to find only one paper(1) on the influence of salts on the activity of adrenalin; this one deals with the inhibition of the pupil reaction.

METHOD.

The usual procedure was employed in obtaining blood-pressure records from the cat--the canula was inserted into the carotid artery and connected to a mercury manometer; a saturated solution of sodium carbonate was used as a transmission fluid. In the first few experiments the test solutions were injected into the femoral vein with a hypodermic syringe, but because of the rapid clogging of the vessel from clotting as a result of this method, a glass canula was tied into the vein and the injections made thru this. The latter method was found to be quite satisfactory in every respect.

In preparing the frog for the experiment the animal was first anesthetized with ether, then fastened onto the holder (Fig.1, Plate I) with ordinary binding tape, after which an occasional drop of ether was sufficient to prevent struggling. The lower part of the abdominal aorta and the two iliac arteries were exposed by removing the os coccygis (see Schulz, loc.cit.)after which the canula (three-way for the purpose of cleaning, Fig.2) was

(1) FRANKL: PFLÜGER'S ARCH., 19 , CXXX, 346-352.

inserted into the right iliac when this vessel was large enough, otherwise the abdominal aorta itself was employed. The canula was connected to a Hürtle manometer, using as a transmission fluid the solution suggested by Schulz--10% dextrose and 1% ammonium oxalate. The injections were made into the crural vein of the left leg thru a glass canula which was tied into this vessel and supported by a clamp. It is necessary to support both canulas in a favorable position because of the great tenderness of the frog's blood-vessels. Thruout the experiment the animal was kept covered with a moist cloth to prevent drying of the skin and other undesirable sensory stimuli, which according to Schulz cause a marked change in the blood-pressure.

The respiratory curve was obtained by the use of a double tambour (Fig. 3) --one placed under the animal's head and in contact with the skin over the submental muscles; the other placed on a stand and operating a writing lever. The results obtained were, however, very unsatisfactory because of the tendency of the frog to suspend respiration at intervals when disturbed.

In preparing the salts for injection volumetric solutions (molecular where the solubility permitted) of chemically pure substances were made , using double distilled water as a solvent. Before administration these were diluted to the desired concentration with double distilled water. The stock solution of adrenalin was made from Parke, Davis and Co's base -- 1 mgm. to each cubic centimeter of water acidulated with 0.004% HCl -- and was discarded when two weeks old.

Frogs of about 100 gm weight were chosen so that a constant dose of the solutions (0.25 c.c.) could be used; this was also true of the cats; animals of practically the same weight were selected and 1 c.c. of solution determined upon as a dose.

RESULTS. Adrenalin on cat.

The first object of this research--investigating frog's blood-pressure as a means of standardizing adrenalin --will be discussed after the salt effects on the blood-pressure have been considered, because of its dependence to a greater or less extent upon the latter.

As this work deals mainly with the action of adrenalin in combination with other substances, a detailed account of the effect of adrenalin alone would be proper at the outset of this discussion of results.

When injected intravenously adrenalin acts very quickly, a maximal rise in pressure taking place within a few seconds, succeeded by a return to normal in a few seconds more -- the whole effect lasting on an average slightly less than one minute (Plate II, Fig. 1). But the interesting phase of the action of adrenalin is its after effect; the curve does not stop when it has reached normal pressure after the rise, but continues to fall till the manometer has shown a decrease equal to or greater than the original rise. I have been unable to find any such action recorded in literature dealing with adrenalin. Lohmann(1) showed that, in certain admixtures of adrenalin and cholin, both

(1) Lohmann, Pflüger's Arch., 1907, 118, 215-227.

~~both~~ substances might act separately -- the adrenalin causing a rise in pressure followed by the cholin depression. To prove that such was not the case in these experiments, a 1:10,000 solution of adrenalin was made alkaline and allowed to stand for several days, when it was found by chemical tests to be completely oxidized; this solution upon injection produced no change in blood-pressure, proving the absence of cholin. After preparation of this paper Loewi found the after depression of adrenalin (2)

RESULTS. Salts and adrenalin on cat.

This after effect of adrenalin caused me to look for a substance which, when injected simultaneously with it, might prevent this undesirable result without in any way diminishing the initial action of the adrenalin. This action was found to result from the administration of barium chlorid. In even as great dilution as a 1/128 M solution this substance injected alone will cause a marked stimulation of the heart action and a rise in blood-pressure (Plate II, Fig. 2). When injected with adrenalin the rise caused by the latter is noticeably augmented, the pressure does not fall so rapidly, and the after depression is entirely absent. The strengthened heart beat may be observed for some fifteen minutes or mere after injection. (Plate II, Fig. 3).

In order to determine whether the mere addition of a given amount of liquid (in these experiments 1 cc. of each salt used) to the circulation would in itself cause a change in pressure, several injections of Ringer's solution(1) were made and were found to produce no effect. The same statement holds concerning the effect of Ringer's solution on adrenalin pressure.

The next salt experimented with was NaCl. Injection of Ringer's solution: NaCl, 0.7%; $CaCl_2$, 0.026%; KCl, 0.03%.

Von Leusum C. Pflügers Archiv. 1911 - p. 377.

an M/8 solution (which is of nearly physiological osmotic pressure) caused no change either in blood-pressure or heart action; and an M/2 solution produced only a slight rise in blood-pressure; these results corroborate the findings of Hyde(1), Mayor(2), and Ritter(3). Nevertheless, from this work, NaCl in the doses given may be considered a neutral salt. But when injected (M/2) with adrenalin it caused a decrease in the original rise; it did not, however, affect the length of action nor did it prevent the after fall which usually follows the adrenalin rise. (See tables I + II)

Potassium chlorid in dilutions of M/32 may be considered practically neutral both alone and in combination with adrenalin Twice as concentrated solutions, however, have a depressing effect on blood-pressure and heart rate when given alone, (Plate II, Fig. 4). and just the opposite effect in connection with adrenalin. (Plate III, Fig. 5). This latter result may be explained by the statement of Howell(4), that KCl increases vagus irritability in certain doses while in larger ones it depressee; the dose of KCl in connection with adrenalin was perhaps sufficient to depress the vagus, while alone it acted as a vagus stimulant, therefore slightly lowering the blood-pressure. (Plate II, Fig. 6)

Calcium chlorid and magnesium sulfat have a depressing effect on blood-pressure both when given alone and when administered with adrenalin. Meltzer and Auer(5) obtained the

(1) Hyde: 1908, Am. Jour. Physiol., Vol.23, 201.

(2) Mayor: 1902, Journ. de Physiol., IV, (3, p.425.

(3) Ritter: 1910, Deutsch.Arch.f.klin.Med.,Cl/2,S 11.

(4) Howell: 1906, Am. Jour. Physiol., V. 15, 280-294.

(5) Meltzer and Auer: 1906, Am. J. Phys., V. 15, 387.

same results from the injection of magnesium, but they used much larger doses, causing complete inhibition of the respiration as well as a fall in blood-pressure.

Some experiments were undertaken with the phosphates as the result of a test which showed the adrenalin base (P.D.& Co) to contain traces of these substances. The phosphates used were the acid, neutral and alkaline sodium salts. No definite effect could be observed either when used alone or when given with adrenalin; the acid phosphate, however, increased the pressure somewhat but had no effect on the adrenalin rise, while the neutral and alkaline salts were without effect when used alone and only the alkaline one, Na_3PO_4, caused any change when given with adrenalin; the change produced here was a marked depression sometimes even complete neutralization of the adrenalin; but this was probably due to oxidation of the adrenalin(which takes place readily in an alkaline solution) rather than to any specific effect of the Na_3PO_4 itself.

Another substance, which, being a waste product in the body, and hence normally coming into contact with other constituents of the blood, would be of interest in this connection, is the purin derivative, uric acid. This compound alone causes a decided rise in mammalian blood-pressure. (Plate III, Fig. 1). Its effect on heart action, however, is very insignificant, being in a majority of cases quite neutral, in others causing only a slight increase in heart rate. It exhibits a peculiarity when injected with adrenalin in that it causes a decrease in the normal adrenalin rise. Just why this seemingly papadoxical result should follow the combination of these two body products,

will probably not be understood until the action within the body of certain substances produced in normal metabolism upon the internal secretions, individually and in co-operation with each other is better known.

Control injections of HCl and NaOH of equal ~~strength~~ acidity and alkalinity to that of M/16 NaH_2PO_4 and Na_3PO_4 respectively, showed that the results obtained from these salts were not due to their reactions. The HCl caused a slight depression while the NaOH had practically no effect upon the blood-pressure. Acid solutions equal in strength to the acidity of 1:10,000 adrenalin had no effect whatever; an alkaline solution of equimolecular concentration was also neutral.

RESULTS. Frog.

The first object of this research -- investigating frog's blood-pressure as a means of standardizing adrenalin -- will be discussed after the salt effects on the blood-pressure have been considered, because of its dependence to a greater or less extent upon the latter.

zur Hervorrufung von Vaguskurven gar nicht einmal nötig, durch auffallende Bewegung in der Nähe der Augen einen Reiz auszuüben, sondern es genügte das Erscheinen eines fremden Gegenstandes innerhalb des Gesichtsfeldes schon auf weite Entfernung hin". In view of this statement and because it is difficult to avoid slight changes in light and other elements of the frog's visual field, I took pains to make the following test on several occasions, both before any injection had been made and

will probably not be understood until the action within the body of certain substances produced in normal metabolism upon the internal secretions, individually and in co-operation with each other is better known.

Control injections of HCl and NaOH of equal ~~strength~~ acidity and alkalinity to that of M/16 NaH_2PO_4 and Na_3PO_4 respectively, showed that the results obtained from these salts were not due to their reactions. The HCl caused a slight depression while the NaOH had practically no effect upon the blood-pressure. Acid solutions equal in strength to the acidity of 1:10,000 adrenalin had no effect whatever; an alkaline solution of equimolecular concentration was also neutral.

RESULTS. Frog.

It was found that, because of the peculiar action of adrenalin in the frog, definite results could not be obtained from injection of salts and adrenalin simultaneously, therefore this discussion will deal with the separate action of these two classes of substances.

I had no occasion to test the reflex stimulation of the vagus thru the sensory nerves other than the optic, on the effect of which Schulz(1) makes the following remark: "Es war zur Hervorrufung von Vaguskurven gar nicht einmal nötig, durch auffallende Bewegung in der Nähe der Augen einen Reiz auszuüben, sondern es genügte das Erscheinen eines fremden Gegenstandes innerhalb des Gesichtsfeldes schon auf weite Entfernung hin". In view of this statement and because it is difficult to avoid slight changes in light and other elements of the frog's visual field, I took pains to make the following test on several occasions, both before any injection had been made and

also between injections. The moist cloth with which the animal was kept covered, as before stated, was lifted and the eye exposed to the light of a 16 candle power electric bulb for one minute at a time; with even this strong stimulation there was absolutely no change in the pressure curve in any experiment. One would expect, however, to observe a slight change in heart action due to reflex vagus stimulation, and in a few instances there was a slowing of one to four beats per minute; but this was by no means a constant result of the light stimulation.

A comparison of tables I and III, pertaining to the effect of salts, will show that when the doses were properly regulated, the salts under consideration had practically the same effect on the frog as on the cat (Plate III, Figs. 2, 3, 4 & 5). With two exceptions -- KCl and $BaCl_2$ -- equimolecular solutions were used on both animals to produce these similar effects, the dose being varied as to quantity only (0.25 c.c. for the frog and 1 c.c. for the cat); of the two salts mentioned, a stronger solution of KCl was required to bring about a depression in the frog's blood-pressure, while a much weaker one of $BaCl_2$ was necessary to produce the heart stimulation and rise in pressure. M/32 barium chlorid was toxic to the frog and would quite often entirely stop the heart for several seconds; M/128 $BaCl_2$ had the usual tonic effect.

It was in working out the initial object of this research (that of determining the value of the frog's blood-pressure as a means of standardizing adrenalin) that obstacles were encountered which would seem to rule out the frog as a reliable animal for this kind of work. The initial dose of

adrenalin would always cause a considerable rise in pressure-- (Plate III, Fig. 2) sometimes as high as 12-15 mm.-- but the succeeding injections declined rapidly till the fourth or fifth dose which would cause a change of probably 1-3 mm., this effect remaining nearly constant for several doses, the size of the dose (The dose used were 0.25 c.c. of 1:100,000, 1:60,000, 1:30,000 and 1:10,000) thereafter making but little difference. The rise in pressure lasted considerably longer than in the cat (see Plate , Fig.) and there was no after depression, but the peculiar decrease in susceptibility to the drug rendered it impossible to determine with any degree of accuracy the strength of adrenalin used, or in the other part of the research, to ascertain the effect of the salts upon adrenalin pressure.

This work was done at the suggestion and under the supervision of Dr. Ida H. Hyde, head of the Physiology Department of this University.

and sustains the high blood-pressure for some time.

2. The waste product, uric acid, antagonizes adrenalin ~~pressure~~, altho alone it increases blood-pressure.

3. Effect of salts used on blood-pressure in the cat and the frog are practically the same.

4. Visual stimulation, even to the strength of a 16 c.p. light, had no effect on blood-pressure of the frog.

5. The simplicity of the technique for obtaining blood-pressure in the frog makes this a desirable animal for experimental purposes, altho it is not practical for use with adrenalin.

6. The frog reacts peculiarly to adrenalin, losing its susceptibility to a marked degree after three or four

adrenalin would always cause a considerable rise in pressure-- (Plate III, Fig. 2) sometimes as high as 12-15 mm.-- but the succeeding injections declined rapidly till the fourth or fifth dose which would cause a change of probably 1-3 mm., this effect remaining nearly constant for several doses, the size of the dose (The dose used were 0.25 c.c. of 1:100,000, 1:60,000, 1:30,000 and 1:10,000) thereafter making but little difference. The rise in pressure lasted considerably longer than in the cat (see Plate , Fig.) and there was no after depression, but the peculiar decrease in susceptibility to the drug rendered it impossible to determine with any degree of accuracy the strength of adrenalin used, or in the other part of the research, to ascertain the effect of the salts upon adrenalin pressure.

CONCLUSIONS.

1. Adrenalin has an after depressing effect on mammalian (cat) blood-pressure, which, considered therapeutically, is very undesirable. This may be counteracted by very small doses of barium chlorid, which also strengthens heart action and sustains the high blood-pressure for some time.

2. The waste product, uric acid, antagonizes adrenalin ~~pressure~~, altho alone it increases blood-pressure.

3. Effect of salts used on blood-pressure in the cat and the frog are practically the same.

4. Visual stimulation, even to the strength of a 16 c.p. light, had no effect on blood-pressure of the frog.

5. The simplicity of the technique for obtaining blood-pressure in the frog makes this a desirable animal for experimental purposes, altho it is not practical for use with adrenalin.

6. The frog reacts peculiarly to adrenalin, losing its susceptibility to a marked degree after three or four

injections, at which time it is also but slightly affected by the size of the dose.

7. The salts which cause a rise in pressure are: NaCl, M/2; $BaCl_2$, M/128 and M/32 (the latter strength acting inthis way only in the cat); Uric acid; and NaH_2PO_4, M/16. Those depressing the pressure: KCl, M/16 and M/32 (not in the frog); $CaCl_2$, M/16; $MgSO_4$, M/8. Those which showed no definite action are: Ringer's solution; NaCl, M/8; $CaCl_2$, M/32; Na_2HPO_4, M/16.

8. Salts increasing adrenalin pressure: KCl, M/16; $BaCl_2$, M/128 and M/32. Those depressing adrenalin effect: NaCl, M/2; $CaCl_2$, M/16; $MgSO_4$, M/8; and Uric acid.

TABLE NO.I. CAT -- SALT EFFECTS.

Solution	Strength	Amt. of salt injected	Effect on blood-press.	Effect on heart action
Adrenalin	1:10,000	0.01 mgm	ave. 64 mm.	rate decr., force greatly increased
Ringer			neutral	none
NaCl	M/8	7.3 mgm	"	"
NaCl	M/2	29.0 "	slight incr. (ave. 2 mm)	undetermined
KCl	M/32	2.2 "	slight decr. (ave. 2 mm)	"
KCl	M/16	4.4 "	decreased (ave. 4 mm)	rate slightly decreased
$CaCl_2$	M/32	3.4 "	neutral	none
$CaCl_2$	M/16	6.8 "	slight decr. (ave. 4 mm)	not determinable*
$MgSO_4$	M/8	15.0 "	decrease (ave. 9 mm)	none or slight incr. in rate
$BaCl_2$	M/128	0.4 "	marked incr. (ave. 15 mm)	rate sl. decr.; force incr.
$BaCl_2$	M/32	1.6 "	marked incr. (ave. 23 mm)	rate markedly decr. force incr.
Uric acid	0.005%	0.01 "	increase (ave. 9 mm)	none or slight incr. in rate

* Rate sometimes slightly increased and sometimes slightly decreased.

TABLE NO. I (Cont).

NaH_2PO_4	M/16	6.0	mgm	slight incr. (ave. 5 mm)	none
Na_2HPO_4	M/16	8.8	"	neutral	"
Na_3PO_4	M/16	10.0	"	variable	rate sl. decreased
HCl*	0.35%			very slight increase	rate sl. decreased
NaOH**	0.3%			neutral or very sl. incr.	rate sl. decr.

* Same acidity as M/16 NaH_2PO_4.

** " alkalinity as M/16 Na_3PO_4.

TABLE NO. II. ADRENALIN AND SALTS -- CAT.

Amount injected -- 1 Cc. each of adrenalin and salt solution.

Adren.*& following	Strength	Effect on Adren. Pressure	Effect on heart Action
Adrenalin alone	1:100,000	ave. 35 mm. Hg.	rate decreased; force increased
"	1:10,000	ave. 64 mm. Hg.	same as above but more marked
Ringer	--------	no change	same as adrenalin alone
NaCl	M/8	variable	" " " "
"	M/2	decr.(ave.5 mm)	" " " "
KCl	M/32	no change	" " " "
"	M/16	incr.(ave.4 mm)	rate and force both decr.
$CaCl_2$	M/16	decr.(" 9 mm)	same as adrenalin alone
$MgSO_4$	M/8.	decr.(" 13 mm)	force incr. less than by adrenalin alone
$BaCl_2$	M/128	incr.(" 9 mm)	augments adren. effect
"	M/32	incr.(" 25 mm)	more marked than M/128
Uric acid	0.005%	decr.(" 12 mm)	rate and force decr.
NaH_2PO_4	M/16	no change	same effect as adrenalin
Na_2HPO_4	M/16	" "	" " " "
Na_3PO_4	M/16	decr.(ave.17 mm)**	" " " "
HCl	0.004%	no change	
NaOH	≠ 0.004% HCl	" "	" " " "

* Strength of adrenalin used was in nearly all cases 1:10,000.

** This marked effect was probably due to oxidation of the adrenalin; which takes place rapidly in alkaline solution

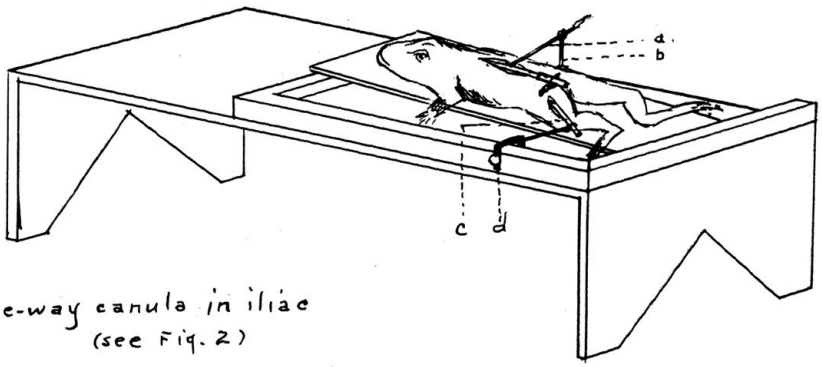
TABLE NO. III. SALT EFFECTS ON FROG.

Amount injected -- 0.25 Cc. of each solution.

Solution	Strength	Amt. of salt injected	Effect on blood-press.	Effect on heart action
Ringer			neutral	none
NaCl	M/8	1.8 mgm.	"	"
"	M/2	7.3 "	"	" or slight decr. in rate
"	M/1	14.6 "	decr. (ave. 9mm)	"
KCl	M/16	1.1 "	neutral	none
"	M/8	2.2 "	marked decr.	nearly stopped for few seconds
$CaCl_2$	M/16	1.7 "	decreased (ave. 4 mm)	rate sl. decr.
$MgSO_4$	M/8	3.75 "	decr. (-3 mm)	none
$BaCl_2$	M/128	0.4 "	incr. (3 mm)	rate decr.; force increased
"	M/32	1.6 "	decr. (-8 mm)	same as M/128 but more marked *
Uric acid	0.005%	0.01 "	incr. (1 mm)	none
NaH_2PO_4	M/16	1.75 "	variable	variable
NaH_2PO_4	M/16	1.75 "	decr. (-4 mm)	"
Na_3PO_4	M/16	2.5 "	" "	rate sl. incr.
HCl	0.004%		sl. incr. (ave. 1 mm)	none
NaOH	0.007%		neutral	"

* This was after the initial toxic effect had passed.

PLATE. I.



a- Three-way canula in iliac (see Fig. 2)
b- Clamp on a.
c- Straight canula in crural.
d- Clamp on same.

FIG. 1.

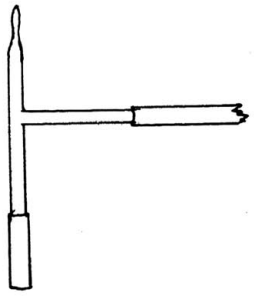


FIG 2.
THREE-WAY CANULA.

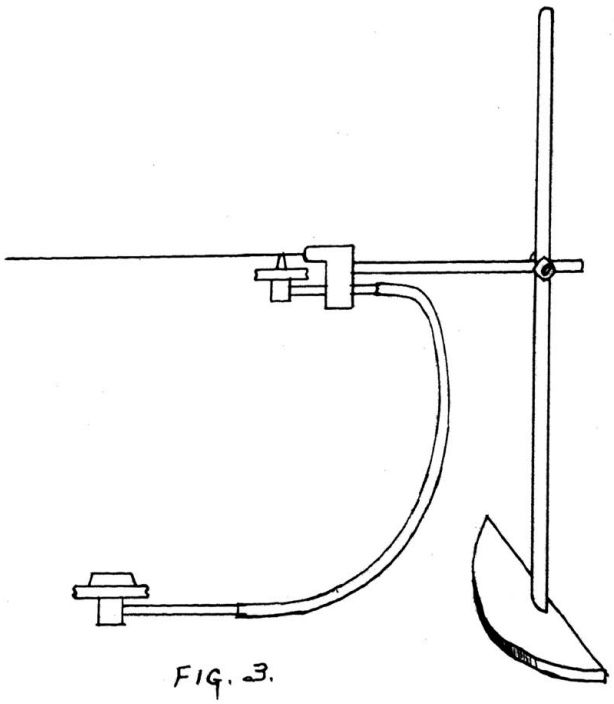


FIG. 3.
RESPIRATORY TAMBOURS.

PLATE II. - FROG.

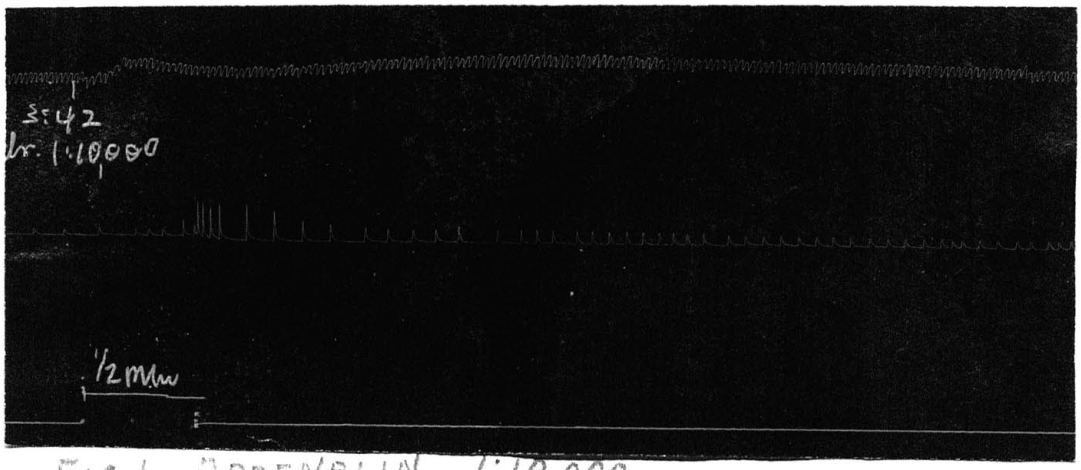


FIG 1. ADRENALIN 1:10,000.

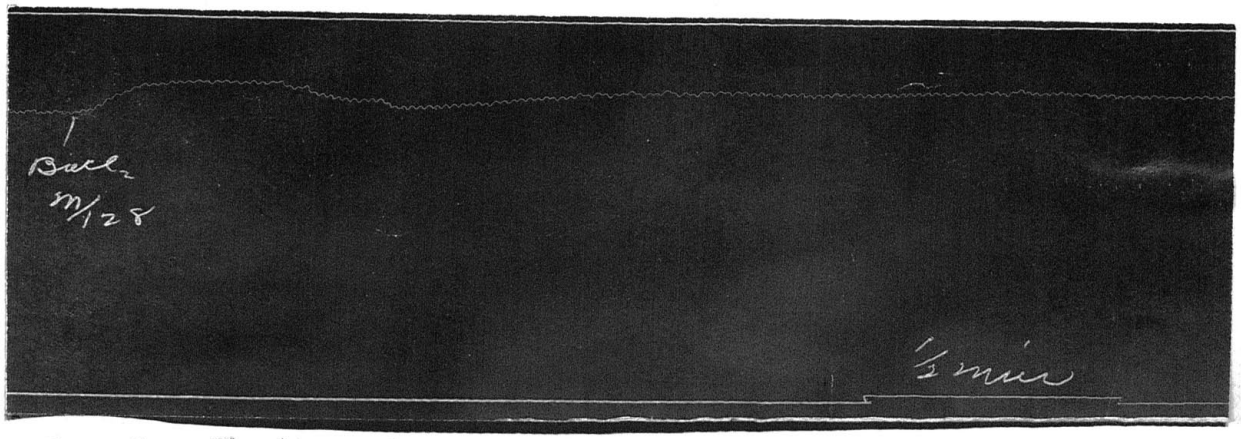


FIG. 2. $BaCl_2$ m/128.

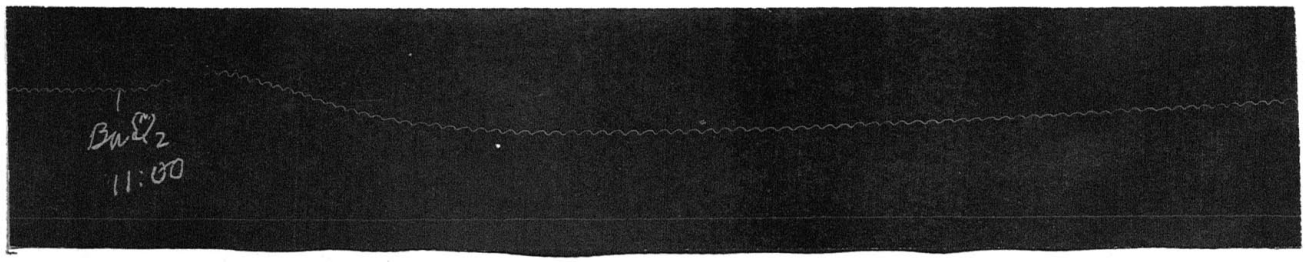


FIG. 3. $BaCl_2$ m/32.

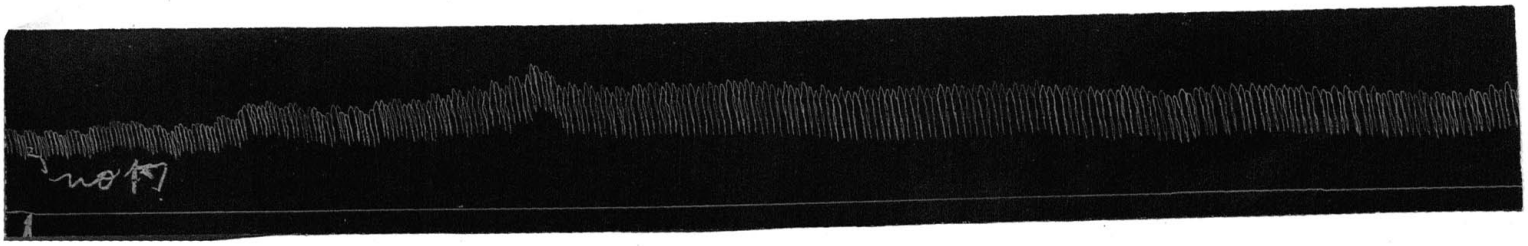
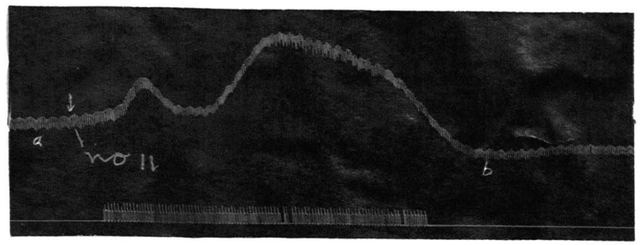


FIG. 3. KCl. m/8.

PLATE II. (CAT).

ARROW SHOWS WHERE INJECTION WAS MADE.

FIG. 1.

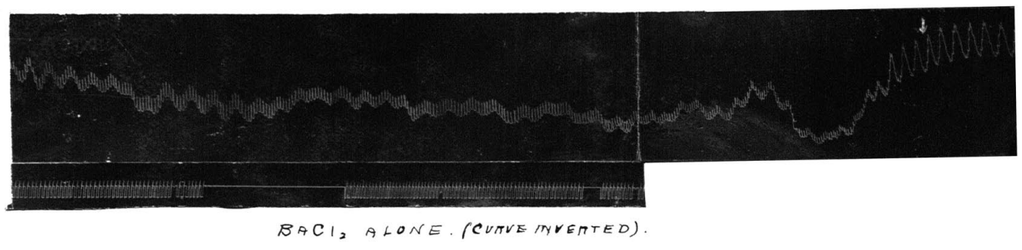


ADRENALIN ALONE.

a - NORMAL CURVE. b - SHOWING AFTER-DEPRESSION.

TIME - ½ SEC.

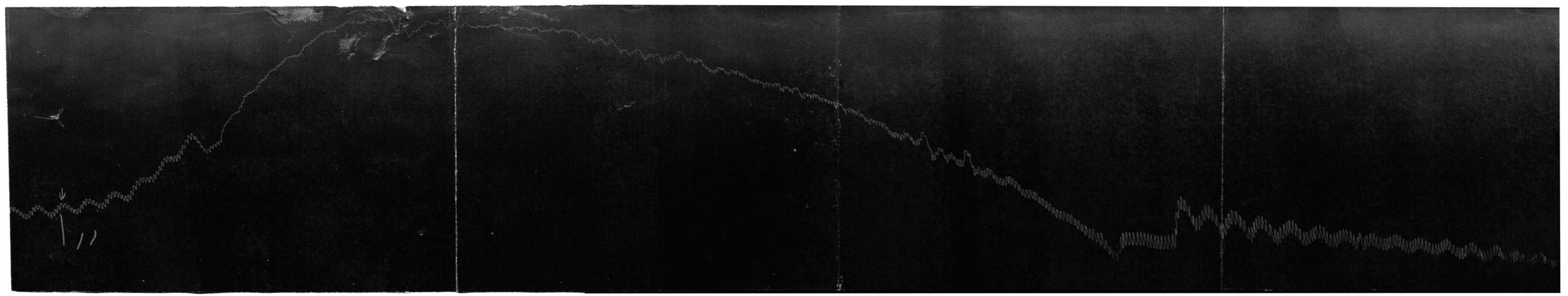
FIG. 2.



$BaCl_2$ ALONE. (CURVE INVERTED).

TIME - ½ SEC.

FIG. 3.

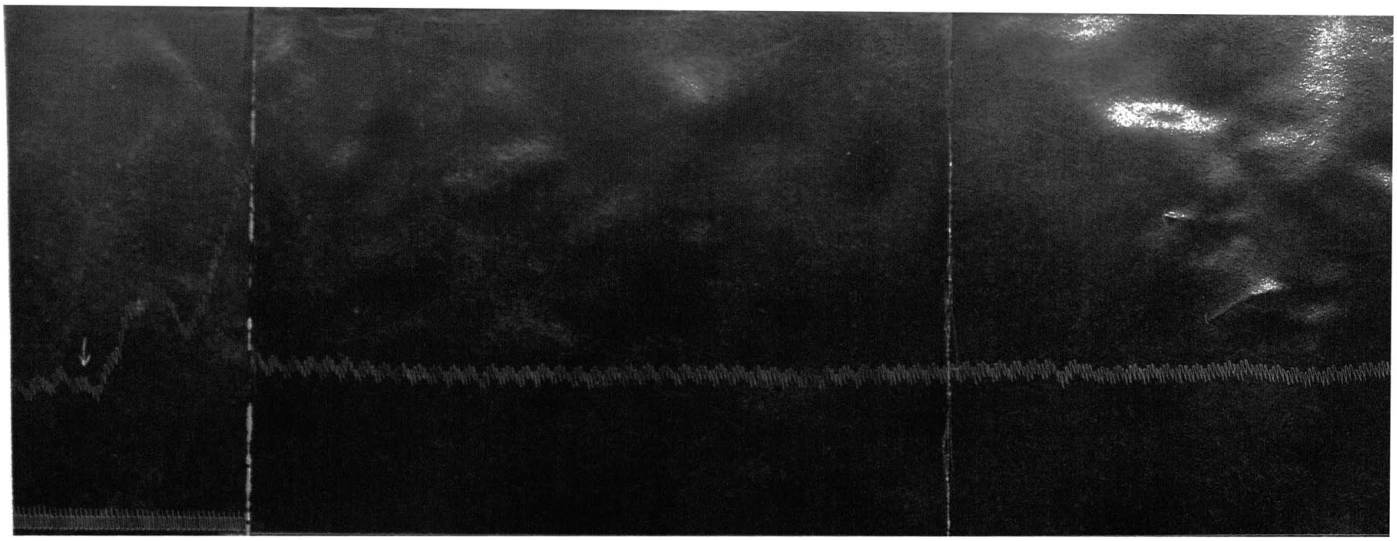


KCl and ADRENALIN.

TIME - SAME RATE OF DRUM AS ABOVE.

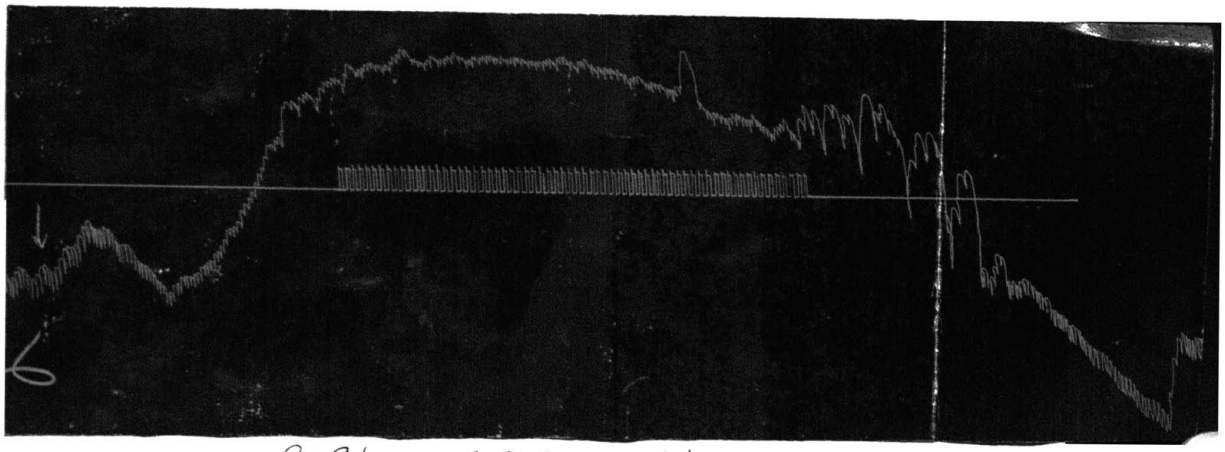
PLATE II (CONT).

Fig. 3.



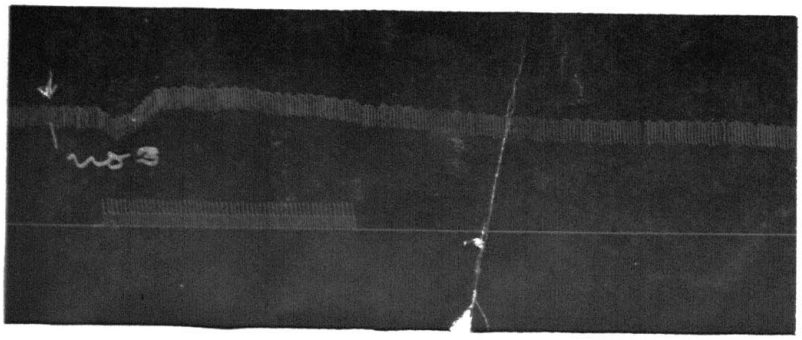
$BaCl_2$ and Adrenalin.

Fig. 5.



$CaCl_2$ and Adrenalin.

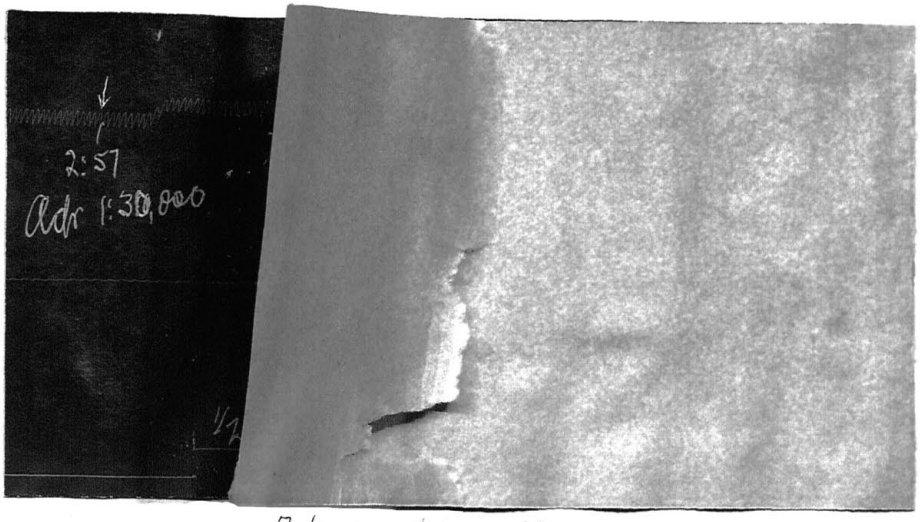
Fig. 6.



Uric Acid.

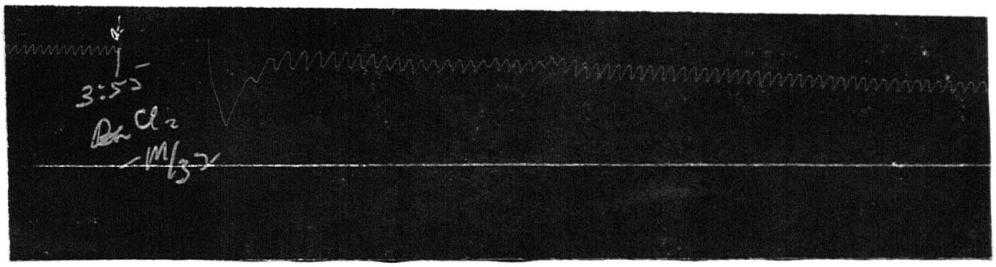
PLATE III. (FROG).

FIG. 1.



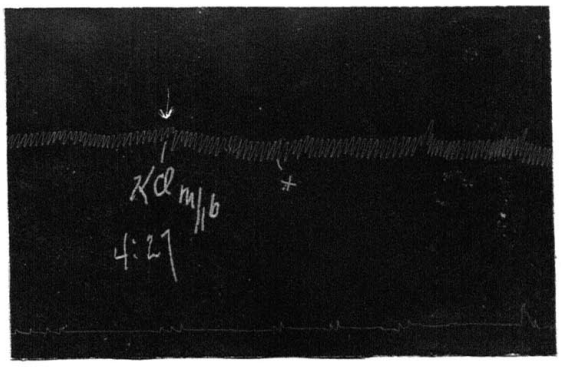
Adrenalin. 1:30,000.

FIG. 2



$BaCl_2$ ALONE.

FIG. 3.



KCl alone.